CHLORIDE EXCRETION IN THE HEAD REGION OF FUNDULUS HETEROCLITUS

JEAN BURNS AND D. EUGENE COPELAND

Arnold Biological Laboratory, Brown University, Providence, Rhode Island

The ability of marine teleosts to actively excrete salts from the head region was first demonstrated by Smith (1930). Although cautious in his statement, he assumed the function to be localized in the gill structure. The perfusion experiments of Keys (1931), Bateman and Keys (1932) and Schlieper (1933) proved that the gill did indeed have that function.

The identity of the cell responsible for the chloride transfer was first suggested by Keys and Willmer (1932). They gave no proof of its function, but made deductions to that end. Bevelander (1935 and 1936), after a comparative survey of many fish gills, was inclined to the view that the general respiratory epithelium, rather than any special cells, was responsible.

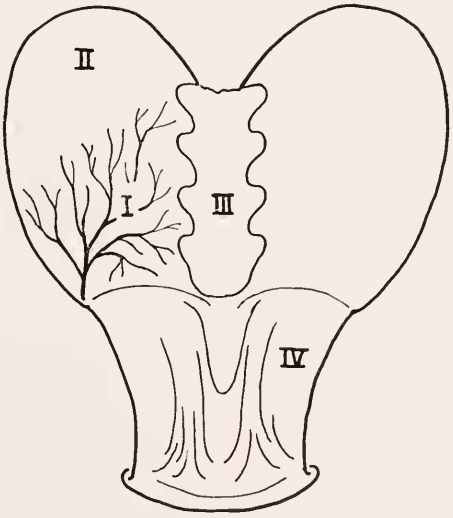


FIGURE 1. A diagram of the lower jaw with the gills removed and the opercula spread out. The normal, gross vascular supply of the operculum is shown on one side (I). The rest of the operculum (II) is relatively poorly supplied. Other areas investigated are the region of gill attachment (III) and the lateral floor of the mouth (IV).

The first significant experimental evidence was furnished by Liu (1942) who found that a certain cell type in the fresh water paradise fish, *Macropodus opercularis,* hypertrophied when the fish was exposed to gradually increased salinities. Later, Copeland (1948) gave experimental evidence that in the euryhaline *Fundulus heteroclitus* the cell homologous to those described by Keys and Willmer (1932)

and Liu (1942) is the cell responsible for chloride excretion in the gill. It has also been suggested (Pettengill and Copeland, 1948) that the same cell can reverse its polarity and be responsible for the chloride absorption observed in fresh water conditions (Krogh, 1937).

Since the chloride excreting cell of *Fundulus heteroclitus* has been investigated and identified by a number of techniques, it was decided to apply a number of them to the rest of the epithelium of the head cavities, both oral and pharyngeal, to see if the special cell is confined to the gill.

Materials and Methods

The fish used for this study were adult *Fundulus heteroclitus* which had been fully adapted to sea water.

They were killed by decapitation. The lower jaw and opercula were separated from the rest of the head and pinned down. Samples of epithelium from the numbered areas of Figure 1 were stripped from the underlying connective tissue and fixed.

Osmiophilia preparations were made following the directions of Ludford (1926).

The mitochondria were demonstrated by the method outlined by Copeland (1948) with the substitution of the following for his picric acid differentiation.

1. Differentiate with 1% methyl green, 5 sec.
2. Wash in distilled water, 10 sec.
3. Rinse in 95% alcohol, 5 sec.
4. Place in absolute alcohol I, 10 sec.
5. Finish dehydration in absolute alcohol II, 3–4 min.
6. Clear in toluene, mount in clarite.

Some of the tissue was fixed in cold 1 : 1 acetone-absolute alcohol and 7 micron sections of this material were incubated at 37° C. in 0.4% Ca glycerophosphate and handled according to Gomori's (1939) directions for the demonstration of alkaline phosphatase.

Results

Chloride cells are found distributed throughout the epithelium of the head cavity, the distribution of the cells showing a distinct correlation with the vascularity of the tissue.

Explanation of Figures

Figure 2. Preparation by the Ludford technique to show osmiophilia.

Figure 3. Alkaline phosphatase preparation by the Gomori technique.

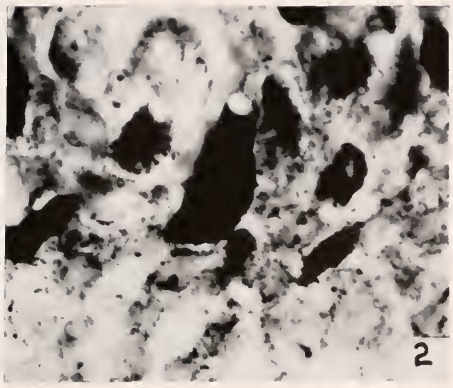
Figure 4. Mitochondrial preparation by the Regaud-Altmann acid fuchsin technique.

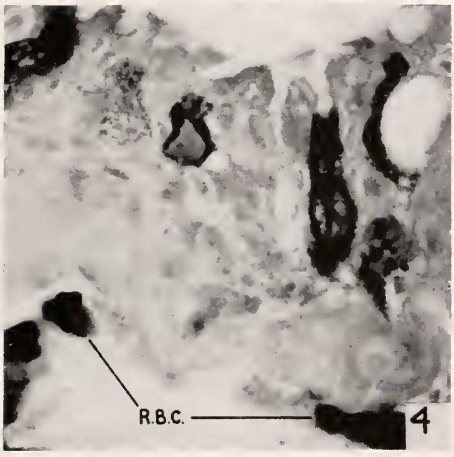
Figure 5. Epithelium from a relatively avascular region (area II of Fig. 1). No chloride cells visible in this particular view, though they are found sparsely scattered in the region. The granular cells are eosinophil granulocytes. Mitochondrial preparation.

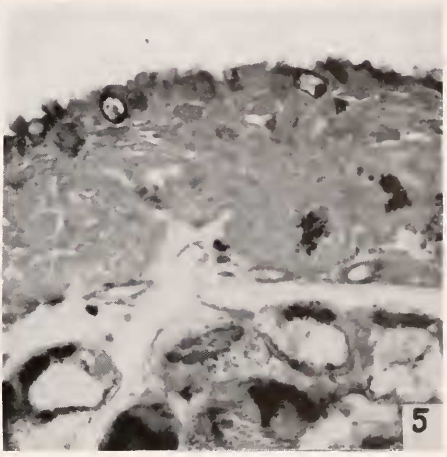
Figure 6. Epithelium from a well vascularized region (area I). Note the number of chloride cells and the proximity of the circulation. Mitochondrial preparation.

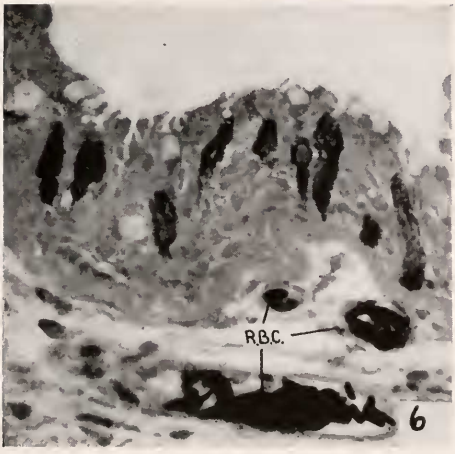
Figure 7. Epithelium from the same area as Figure 5, but from an animal that had an abnormal degree of vascularization in that area. An accompanying density of chloride cells is found. Mitochondrial preparation.

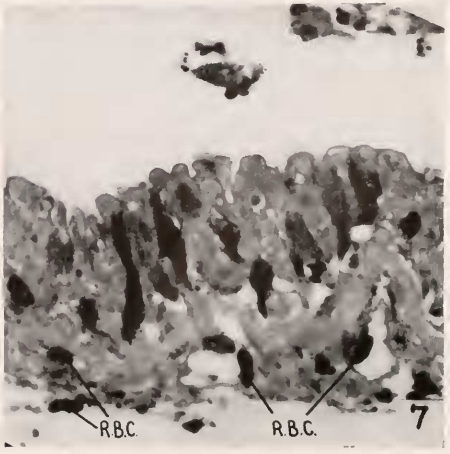
Magnification: Figures 2, 3, 4 approximately 800 × (oil immersion) and 5, 6, 7 approximately 400 × (high dry).











FIGURES 2–7

The morphology of the cells is like that of the chloride cells of the gills. The osmiophilic (Fig. 2) and alkaline phosphatase (Fig. 3) pictures correspond to those shown of the gill chloride cells by Copeland (1950) and Pettingill and Copeland (1948). The mitochondria pattern (Fig. 4) is much like that in the gills (Copeland, 1948). One dissimilarity can be noted: the cells of the gills usually contain a greater density of mitochondria.

Chloride cells are found in all parts of the cavity. In areas II, III, and IV of Figure 1 and in the roof of the mouth they are sparsely distributed, histological preparations showing many sections devoid of cells (Fig. 5). In area I they are almost as numerous as in the gill epithelium (Fig. 6).

The correlation between number of chloride cells and degree of vascularity of the epithelium is very marked. In an area of good vascularity the cells are always present in abundance (area I). The reverse is seen in regions of poor vascularity (areas II, III, IV and the roof of the mouth). That a relation exists between the cell population and vascularity of the tissue is indicated by the following observation. In one case the grossly visible blood supply extended to an unusual degree into a normally "avascular" region (area II). Histological examination revealed a large number of cells where normally few are found (Fig. 7).

Discussion

The chloride excreting mechanism in the head region of teleosts has a much wider basis than originally assumed. Chloride excreting cells similar to those in the gill filaments are found in the rest of the oral, pharyngeal, and opercular epithelium. The number in the oral cavity and in the region of the gill bar attachment (pharnygeal) is probably not great enough to play a significant part in osmoregulation. However, the relatively huge number in the opercular lining is significant.

It is very interesting that there is a correlation between the number of cells and the degree of vascularity seen in the operculum. Since the cells presumably control the salt level of the body through the mediation of the circulatory system, it is significant that such a correlation is found.

Keys and Willmer (1932) calculate that in a 250 gram eel there are 3 to 6 million chloride cells present in the gill, enough to account for the observed salt excretion. Assuming the two teleosts to be homologous in this basic arrangement, the discovery of additional cells increases the adequacy of the tissue as a mechanism for extra-renal osmoregulation.

Summary

1. The chloride excreting cell is not limited to the gill epithelium, being found in other regions of the head, especially the inner surface of the operculum.

2. With one exception, the cells appear identical in morphology and in response to Regand-Altmann, Ludford, and alkaline phosphatase techniques. The exception is that the mitochondria are usually more densely packed in the branchial cells.

3. In the operculum, the population density of the chloride cells is in positive ratio to the vascularity of the tissue. Such a topographical positioning of the cell is significant to its function of removing chlorides from the circulatory system.

Literature Cited

Bateman, J. B. and A. Keys, 1932. Chloride and vapour-pressure relations in the secretory activity of the gills of the eel. *J. Physiol.*, **75**: 226–240.

Bevelander, Gerrit, 1935. A comparative study of the branchial epithelium in fishes, with special reference to extra-renal excretion. *J. Morph.*, **57**: 335–352.

Bevelander, Gerrit, 1936. Branchial glands in fishes. *J. Morph.*, **59**: 215–224.

Copeland, D. Eugene, 1948. The cytological basis of chloride transfer in the gills of *Fundulus heteroclitus*. *J. Morph.*, **82**: 201–227.

Copeland, D. Eugene, 1950. The adaptive behavior of the chloride cell in the gill of *Fundulus heteroclitus*. *J. Morph.* (in press).

Gomori, G., 1939. Microtechnical demonstration of phosphatase in tissue sections. *Proc. Soc. Exptl. Biol. Med.*, **42**: 23–26.

Keys, Ancel B., 1931. Chloride and water secretion and absorption by the gills of the eel. *Zeit. vergl. Physiol.*, **15**: 364–388.

Keys, Ancel B. and E. N. Willmer, 1932. "Chloride secreting cells" in the gills of fishes, with special reference to the common eel. *J. Physiol.*, **76**: 368–378.

Krogh, A., 1937. Osmotic regulation in fresh water fishes by active absorption of chloride ions. *Zeit. vergl. Physiol.*, **24**: 656–666.

Liu, C. K., 1942. Osmotic regulation and "chloride secreting cells' in the paradise fish, *Macropodus opercularis*. *Sinensia*, **13**: 15–20.

Ludford, R. J., 1926. Further modifications of the osmic acid methods in cytological technique. *J. Roy. Micro. Soc.*, **46**: 107–109.

Pettengill, Olive and D. Eugene Copeland, 1948. Alkaline phosphatase activity in the chloride cell of *Fundulus heteroclitus* and its relation to osmotic work. *J. Exp. Zool.*, **108**: 235–242.

Schlieper, Carl, 1933. Über die osmoregulorische Funktion der Alkiemen. *Zeit. vergl. Physiol.*, **18**: 682–695.

Smith, Homer W., 1930. The absorption and excretion of water and salts by marine teleosts. *Am. J. Physiol.*, **93**: 480–505.